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Citation for published version:
Gripp, KW, Baker, L, Kandula, V, Conard, K, Scavina, M, Napoli, JA, Griffin, GC, Thacker, M, Knox, RG, Clark, GR, Parker, VER, Semple, R, Mirzaa, G & Keppler-Noreuil, KM 2016, 'Nephroblastomatosis or Wilms tumor in a fourth patient with a somatic PIK3CA mutation', *The American Journal of Medical Genetics - Part A (AJMG)*, vol. 170, no. 10, pp. 2559-2569. https://doi.org/10.1002/ajmg.a.37758

Digital Object Identifier (DOI):
10.1002/ajmg.a.37758

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
The American Journal of Medical Genetics - Part A (AJMG)

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Download date: 05. May. 2023

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Journal:	*American Journal of Medical Genetics: Part A*
Manuscript ID	16-0227.R2
Wiley - Manuscript type:	Clinical Report
Date Submitted by the Author:	n/a
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Keywords:	Wilms tumor, nephroblastomatosis, PIK3CA related overgrowth, hemihyperplasia, hemihypertrophy, CLOVES, lipoma, somatic mutation
Search Terms:	

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Nephroblastomatosis or Wilms Tumor in a Fourth Patient with a Somatic *PIK3CA* Mutation

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Abstract

Wilms tumor and nephroblastomatosis are associated with syndromic conditions including hemihyperplasia. Hemihyperplasia is genetically heterogeneous and may be the result of genomic abnormalities seen in Beckwith-Wiedemann syndrome, mosaic chromosome or genomic abnormalities, or somatic point mutations. Somatic missense mutations affecting the PI3K-AKT-MTOR pathway result in segmental overgrowth and are present in numerous benign and malignant tumors. Here we report a fourth patient with asymmetric overgrowth due to a somatic *PIK3CA* mutation who had nephroblastomatosis or Wilms tumor. Similar to two of three reported patients with a somatic *PIK3CA* mutation and renal tumors, he shared a *PIK3CA* mutation affecting codon 1047, presented at birth with asymmetric overgrowth and had fibroadipose overgrowth. Codon 1047 is most commonly affected by somatic mutations in *PIK3CA*-related overgrowth spectrum (PROS). While the fibroadipose overgrowth phenotype appears to be common in individuals with *PIK3CA* mutations at codon 1047, individuals with a clinical diagnosis of Klippel-Trenaunay syndrome or isolated lymphatic malformation also had mutations affecting this amino acid. Screening for Wilms tumor in individuals with PROS-related hemihyperplasia may be considered and, until the natural history is fully elucidated in larger cohort studies, may follow guidelines for Beckwith-Wiedemann syndrome or isolated hemihyperplasia. It is not known if the specific *PIK3CA* mutation, the mosaic distribution or the clinical presentation affect the Wilms tumor or nephroblastomatosis risk in individuals with PROS.

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Key Words: Wilms tumor, nephroblastomatosis, *PIK3CA*-related overgrowth, hemihyperplasia, hemihypertrophy, CLOVES, lipoma, somatic mutation

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INTRODUCTION

Overgrowth syndromes can be associated with an increased risk for malignant tumors. Beckwith-Wiedemann syndrome is a typical example of this association [Shuman et al., 2000]. Asymmetric overgrowth or hemihyperplasia occurs in patients with Beckwith-Wiedemann syndrome (MIM 130650), but can be an isolated finding. An increased risk for malignant tumors, particularly for Wilms tumor, has been documented in individuals with Beckwith-Wiedemann syndrome or isolated hemihyperplasia (MIM 235000). Hemihyperplasia is genetically heterogeneous, including genomic abnormalities seen in Beckwith-Wiedemann syndrome, as well as mosaic chromosome or genomic abnormalities and somatic point mutations. Somatic mutations affecting the PI3K-AKT-MTOR pathway result in segmental overgrowth and other physical findings. Similarly, somatic mutations affecting the PI3K-AKT pathway are present in numerous benign and malignant tumors (see [Samuels and Waldman, 2010] for review). In individuals presenting in early childhood with segmental overgrowth or other findings related to somatic mutations in the PI3K-AKT-MTOR pathway, the mutation inherently occurred during an early developmental stage and may result in an increased lifetime risk for neoplasias driven by mutations in this pathway. Detailed understanding of the clinical phenotypes related to these mutations [Lee et al., 2012; Lindhurst et al., 2012; Poduri et al., 2012; Rios et al., 2012; Riviere et al., 2012; Kurek et al., 2012; Mirzaa et al., 2013; Keppler-Noreuil et al., 2014; Keppler-Noreuil et al., 2015] may allow for delineation of the associated cancer risks based on the specific mutation and the affected cell lineages. Here we report two individuals with somatic mosaicism for the most common *PIK3CA* mutations, c.3140A>G p.His1047Arg and c.3140A>T p.His1047Leu, and a history of Wilms tumor or

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nephroblastomatosis and compare their presentations to the two other reported individuals with a somatic *PIK3CA* mutation and Wilms tumor or nephroblastomatosis.

MATERIALS AND METHODS

Patient 1 was evaluated clinically and testing for overgrowth was completed clinically. Informed signed consent was obtained, and clinical data, clinical photographs and molecular results were reviewed. Patient 2 was enrolled in a somatic overgrowth study and evaluated at the National Institutes of Health after obtaining informed consent. She was previously reported as patient 23 in Keppler-Noreuil et al. [2014]. Updated history, exam and testing were obtained for this report.

We reviewed the literature and reviewed cohort data in order to gather information on the frequency of Wilms tumor and nephroblastomatosis in individuals with somatic *PIK3CA* mutations.

Cambridge cohort

This study was approved by the UK National Research Ethics Committee. Written informed consent was obtained from all participants or their parents. Genomic DNA was extracted from lesions using standard procedures and imaging was conducted as part of routine clinical care. Somatic *PIK3CA* mutations were detected in affected tissues using Next Generation Sequencing with preceding target enrichment. Equipment and materials were purchased from Life Technologies, Thermo-Fisher Scientific using a custom-designed primer pool which provides coverage of all coding regions of *PIK3CA* and related genes (primer sequences available on

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request). The mean depth of coverage for sequencing was 2000X. Mutations were verified to be disease-causing on the basis of; i) finding the same mutation in additional probands with a similar phenotype ii) published experimental data confirming activation of downstream effectors of PI3K, and/or iii) the presence of the mutation in the catalogue of somatic mutations in cancer (COSMIC) [Forbes et al., 2015].

Clinical Reports

Patient 1

The proband was born vaginally at 35 weeks of gestation after a pregnancy complicated by maternal urinary tract infection and possible polyhydramnios on prenatal ultrasound. His G2P1->2 mother was 22 years old and his father was 26 years old. His African American parents were non consanguineous. One maternal and three paternal half-sibs were in good health. Birth weight was 2.47 kg (25-50th centile for gestational age) and length 48.3 cm 75th centile for gestational age). OFC was not documented. Asymmetric overgrowth with right thigh enlargement was present from birth and resulted in evaluations for hemihyperplasia. At age 5 months, physical examination was remarkable for bilateral supranumerary nipples, increased girth in the right leg compared to the left, and a hypopigmented lesion on the lower abdomen.

A small left kidney with enlargement of the right kidney was noted on ultrasound at age 5 weeks. Renal ultrasound at age 9 months showed three well defined hypoechoic avascular masses in the right kidney measuring 1.3x1.1x1.4 cm, 1.6x1.4x1.4 cm and 1.7x1.3x1.4 cm, respectively (Figure 1a). The lesions were confirmed by CT study (Figure 1b, c), which incidentally showed a marked asymmetry of the paravertebral and pelvic musculature with all

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muscles on the right larger than the on the left. The MRI imaging similarly demonstrated the renal masses (Figure 1d). Wilms tumor was diagnosed and chemotherapy administered in an effort to allow surgery later with preservation of kidney function. This decision was in consideration of his atrophic left kidney, which contributed 23% to total renal function. A subsequent CT scan (Fig. 1c) demonstrated the tumors' sizes to be unchanged or increased, and a needle biopsy was performed at age 10 months. In this post-treatment biopsy the pathology diagnosis was a nephrogenic lesion, it was impossible to differentiate between nephrogenic rests and Wilms tumor. Chemotherapy was completed as planned. The lesions responded to therapy and were monitored through imaging studies. Surgical resection was not performed.

A soft lipomatous mass in the right paraspinal region above the iliac crest was first documented at age 2 years. An MRI at age 7 years showed a 2.8x2.8x10 cm focus of abnormal signal intensity within the right vastus lateralis muscle. An MRI at age 7 10/12 years showed the same stable lesion and regions of post contrast enhancement in the right iliac muscle (Figure 2, a-d). Fatty lobules in the left paraspinal soft tissue at L3-L5 appeared stable compared to previous studies and did not encroach on the neural foramina. The right L3 root was anteriorly displaced, implying fatty infiltration in the right L3-L4 neural foramen. Stable fatty prominence in the right L5-S1 neural foramen was noted. Due to discomfort, the paraspinal intramuscular lipoma was surgically removed at age 8 years. Pathology showed mature adipose tissue encompassing skeletal muscle, consistent with an intramuscular lipoma.

The size discrepancy of his legs persisted. An enhancing lesion in the right psoas was seen on MRI (Figure 2b) and a needle biopsy results obtained at age 4 years suggested an inflammatory myopathy. The entire right leg was larger than the left in diameter and the right

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femur was approximately 1 cm longer than the left at age 7 years. The patient had a mild gait abnormality owing to the asymmetric overgrowth. Persistent thigh pain was the indication for an MRI showing signal abnormalities in the vastus lateralis and peroneus brevis (Figure 2 c, d), but open muscle biopsy at age 8 years showed skeletal muscle without significant pathology.

His motor and speech development were age appropriate with walking independently at age 1 year and single words around the same time. At age 7 years his cognitive development was age appropriate and he attended a typical classroom setting. His height at age 7 years was 127 cm (50^{th}-75^{th} centile), weight 33.7 kg (>97^{th} centile, Z-score 1.8) and OFC 52.5 cm (50^{th}-75^{th} centile). His facial features were symmetric and non dysmorphic. A large mass was visible in the right paraspinal region (Figure 3a), and the leg size discrepancy was striking (Figure 3b, c).

Molecular Laboratory Study Results

An overgrowth panel was performed clinically on a next generation platform at the University of Pennsylvania. Testing included site-specific regions for *AKT1, AKT2, AKT3, GNAQ, MTOR, PIK3CA* and *PIKR2;* in addition the coding and flanking intronic boundaries for *CDKN1C* were covered.

DNA samples derived from a frozen psoas muscle tissue contained a mosaic heterozygous (12.9-15.7%) *PIK3CA* mutation, c.3140A>G, in exon 20, predicting a p.His1047Arg amino acid substitution. The same mutation was present as mosaic heterozygote (21.4-24.8%) in a DNA sample from a tissue block of the right thigh mass. Blood sample derived DNA did not show the mutation with a confidence score of 99.99%, leading to the conclusion that the mutation occurred somatically.

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Additional tests performed with non-diagnostic results included methylation-sensitive multiplex ligation-dependent probe amplification for large deletions, duplications and/or methylation abnormalities in the IC1 *(H19)* and IC2 *(LIT1)* critical regions on 11p15 associated with Beckwith-Wiedemann syndrome.

Patient 2

This individual was originally reported as patient 23 in Keppler-Noreuil et al. [2014] and clinical information was updated at age 10 years. The proposita was born at 38 weeks' gestation by induced vaginal delivery after a pregnancy complicated by abnormal prenatal ultrasounds revealing "webbed toes" on her right foot and "white spots" on her heart. Her G1P0- 1 mother was 21 years and her father was 26 years old. Her African American parents were nonconsanguinous and subsequently had a healthy son. Birth weight was 2.95 kg (10-25th centile) and length 49.5 kg (50th centile). Her right leg and foot, including her toes were noted to be enlarged, and she had hyperpigmentation of her neck and waist.

At age 2 years, she was found on renal ultrasound imaging to have bilateral hypodense renal lesions. She was diagnosed with Wilms tumor. She was treated with 4 months of chemotherapy with vincristine, actinomycin, and adriamycin followed by bilateral partial nephrectomies. Pathology from the nephrectomies showed adenomatous nephrogenic rests. Her renal function has been normal. At age 10 years, abdominal ultrasound showed stable size asymmetry of the kidneys with no change in the moderate hydronephrosis involving the right kidney; parenchymal thinning with increased cortical echogenicity of the right kidney, and normal corticomedullary differentiation of the left kidney with an unchanged nephrogenic rest.

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She has had progressive overgrowth of her right leg and foot with leg length discrepancy. She has undergone multiple orthopedic surgeries including right foot Boyd amputation at age 2 years and epiphysiodesis of the right femur and hemiepiphysiodesis of the right tibia at age 4 years. An MRI scan of her lower extremities at 6 years showed diffuse multi-compartmental lipomatosis of the lower extremities with muscular infiltration, right greater than left with right buttock and leg enlargement and muscle atrophy. Right patellar dislocation was present associated with the intra-articular lipomatosis. Liposuction of her right leg was performed at age 6 years, and debulking of her right knee at 7 years. She had eight-plate removal from the right lateral proximal tibia, medial distal femur and lateral distal femur at 7 years. She underwent laparoscopic surgery to remove excess subcutaneous fat from the right side of her abdomen at age 8 years.

Her motor and speech development were apparently normal. She crawled at 6 months, walked at 10 months, drank from a cup at 11 month, spoke in 2 word sentences at 12 months, toilet trained at 14 months, and was riding a 2 wheel bike at 8 years. She was in a regular class setting with additional help in math. She was diagnosed with attention deficit disorder at age 9 years.

On examination at 10 years, her height was 139. 2 cm (50-75th centile), weight 45.4 kg (90-97th centile) and OFC 54.2 cm (85th centile). Tanner III breast development was noted. She had marked increased, asymmetric enlargement of the right leg and right buttock since her initial examination, and a right flank pigmented nevus extending from her waist to her pelvis bilaterally. She had soft tissue overgrowth of her left lower and right abdomen and

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enlargement of her left labia majora. She ambulated with a right Syme prosthesis. Fat tissue appeared reduced at the arms, upper torso, and face with prominent muscles and vasculature. Thigh circumference was right 61 cm, left 38.8 cm; calf circumference: right 37.5 cm, left 25.2 cm. Feet length: right amputated, left 24.6 cm. Right hip was lower than the left. Her left great toe was enlarged and laterally deviated (Figure 4,a-d).

Molecular Laboratory Study Results

Molecular analysis consisted of candidate mutation analysis for somatic mutations in *PIK3CA* using a custom PCR restriction assay as described in Lindhurst et al. [2012] for the c.3140A>T p.His1047Leu mutation. This mutation was found in adipose and skin samples from the left leg at the level of 3-4%, with negative results from peripheral blood.

PTEN mutation analysis was normal.

RESULTS

Literature Review and Cohort Data

Literature review revealed two reports including individuals with a somatic *PIK3CA* mutation and Wilms tumor [Kurek et al., 2012; Luks et al., 2015] (Table I). Keppler-Noreuil et al. [2014] reviewed the clinical and natural history of *PIK3CA* related overgrowth spectrum in a cohort of 35 individuals (Table II), including one reported here with updated history and findings as Patient 2. Further, two patients with Wilms tumor and megalencephaly-capillary malformation (MCAP) (602501) syndrome were reported prior to the identification of the molecular basis of MCAP [Lapunzina et al., 2004; Wright et al., 2009].

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In a combined cohort of 159 individuals with somatic mutations in *PIK3CA* from the Seattle Children's Research Institute and the University of Cambridge, UK, no individual was identified with Wilms tumor or nephroblastomatosis (Table II). However, these individuals had a broad spectrum of clinical phenotypes, including CLOVES syndrome (612918), MCAP, fibroadipose overgrowth and isolated hemihyperplasia or macrodactyly. It is important to note that longitudinal follow-up data are not available on all individuals and formalized tumor screening by abdominal imaging has not been performed on all. The individuals from the UK ranged in age from one to 57 years (mean of 16 years), and 45 individuals have had formalized screening with abdominal imaging (ultrasound, MRI, CT scan). Overlap of the Cambridge cohort with that reported by Keppler-Noreuil et al. [2014] is noted in Table II.

DISCUSSION

The two patients described here were diagnosed with renal masses at 9 months and 2 years of age, respectively. Imaging studies in both identified hypodense masses in the kidneys suggestive of Wilms tumor. In the first patient, pathology from needle biopsy performed after treatment was indeterminate regarding the diagnosis of Wilms tumor versus nephrogenic rests. In the second patient, pathology showed adenomatous nephrogenic rests. Both patients were treated with chemotherapy, and their follow up studies have been stable. Very few patients with hemihyperplasia due to a somatic *PIK3CA* mutation and Wilms tumor or nephroblastomatosis have been reported (Table I). Nephrogenic rests or nephroblastomatosis refer to foci of embryonal cells persisting beyond 36 weeks of gestation and capable of developing into nephroblastomas (Wilms tumor) [Murphy et al., 2004]. These are found in

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approximately 1% of infant kidneys at autopsy and are associated with an increased risk of Wilms tumor [Lonergan et al., 1998]. Nephroblastomatosis is associated with syndromes including Beckwith-Wiedemann syndrome, isolated hemihyperplasia, chromosomal abnormalities and aniridia [Scott et al., 2006a]. These precursors of Wilms tumor are encountered in 25-40% of patients with Wilms tumors. They are often considered a spectrum lesion and, like in Patient 1 reported here, cannot always be distinguished. Perilobar nephroblastomatosis is typically treated with chemotherapeuty, as was done in Patient 1.

Kurek et al. [2012] described a female with a clinical diagnosis of CLOVES syndrome and a history of Wilms tumor. She had lipomatous overgrowth of the trunk and limbs, with wide feet and polydactyly in addition to striking overgrowth of both legs. This patient was mosaic for the PIK3CA p.His1047Arg mutation in her legs, but negative for the mutation in a saliva-derived DNA sample. Another individual with CLOVES and Wilms tumor was mosaic for the PIK3CA p.Asn345Lys mutation and limited clinical information was available (Table I) [Luks et al., 2015]. Neither the individuals reported here nor the patient in Kurek et al. [2012] had megalencephaly. This is noteworthy because the phenotypic spectrum associated with *PIK3CA* mutations encompasses MCAP syndrome, a clinically distinct disorder manifesting predominantly with severe brain overgrowth, and milder body overgrowth than other *PIK3CA*-related disorders [Mirzaa et al., 2013]. In a series of 12 patients with MCAP, one had a Wilms tumor [Wright et al., 2009]. This 4-year-old male patient had lipomas and a dermatomyofibroma. He did not show a *PTEN* mutation, no other testing was reported [Wright et al., 2009]. Another patient with MCAP and Wilms tumor has been reported [patient 2, Lapunzina et al., 2004]. This 10 month old girl had megalencephaly, hydrocephalus, cutaneous

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vascular malformations, joint hyperlaxity, asymmetry and 2-3 toe syndactyly [Lapunzina et al., 2004]. We are not aware whether this patient has been tested for *PIK3CA* mutations.

Somatic *PIK3CA* Mutation Associated Phenotypes

Phenotypes associated with somatic *PIK3CA* mutations are extremely variable, depending upon the timing and location of their postzygotic occurrence and the effect of the specific amino acid on the protein product. This phenotypic spectrum is now referred to as *PIK3CA*-Related Overgrowth Spectrum (PROS) [Mirzaa et al., 2013; Keppler-Noreuil et al., 2014] and encompasses a number of originally clinically defined conditions. The MCAP syndrome was previously known as macrocephaly- cutis marmorata teleanciectasia congenita or macrocephaly-cutis marmorata and is primarily distinguished by brain overgrowth (megalencephaly or hemimegalencephaly) with associated neurologic complications (hydrocephalus, Chiari malformation), cutaneous capillary malformations with focal or generalized somatic overgrowth and syndactyly or polydactyly, as well as variable connective tissue dysplasia [for review see Mirzaa et al., 2013]. The CLOVE syndrome is defined by congenital lipomatous overgrowth, vascular malformations and epidermal nevi [Sapp et al., 2007] and shows significant overlap with fibroadipose hyperplasia. The acronym was extended to CLOVES in order to account for skeletal anomalies, scoliosis, spinal anomalies and seizures [Alomari 2009]. The CLOVES syndrome may be differentiated from MCAP by the severity of somatic overgrowth with characteristic overgrowth of lipomatous tissue and high risk of lymphatic and vascular malformations in the former; whereas brain overgrowth predominates in MCAP and somatic manifestations, while present, are typically milder than in CLOVES

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syndrome. Skeletal anomalies including scoliosis and macrodactyly may be more prominent in CLOVES syndrome, but polydactyly and syndactyly occur in both syndromes. Individuals having overlapping findings of both syndromes are increasingly recognized. Overlap of phenotypic findings between CLOVES syndrome and MCAP is exemplified by the extensive cutaneous involvement of a truncal vascular malformation in Patient 3 reported by Sapp et al., [2007] and the individual reported by Gucev et al. [2008], who had features of CLOVES syndrome and hemimegalencephaly. Vascular malformations affecting the skin in combination with focal overgrowth are characteristic for Klippel-Trenaunay syndrome, and *PIK3CA* mutations were found in 3/15 patients clinically diagnosed with Klippel-Trenaunay syndrome [Kurek et al., 2012]. In the majority of patients with Klippel-Trenaunay syndrome, isolated lymphatic malformation or a combination of findings including fibro-adipose vascular anomalies, mutations in *PIK3CA* were identified [Luks et al., 2015]. A wide range of unusual presentations has been described in case reports, including unilateral hand muscle overgrowth [Castiglioni et al., 2014], segmental overgrowth syndrome [Rasmussen et al., 2014] and mesenteric lipomatosis [Cohen et al., 2014]. The most common mutation is a postzygotic change affecting amino acid 1047, with p.His1047Arg in 19 and p.His1047Leu in 8 of 35 individuals reviewed by Keppler-Noreuil et al. [2014] (Table II). While the CLOVES syndrome or fibroadipose hyperplasia phenotype appears to be common in individuals with a missense mutation at codon 1047, individuals with a clinical diagnosis of Klippel-Trenaunay syndrome or isolated lymphatic malformation [Kurek et al., 2012; Luks et al., 2015] also had a mutation affecting this amino acid. Keppler-Noreuil et al. [2014] differentiated between mutations at p.His1047, which affect the catalytic domain of the protein product, and multiple other changes in the coiled domain

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and concluded that the majority of patients with CLOVES syndrome had mutations in the latter. While this was statistically significant within their cohort, there were exceptions even within their relatively small cohort.

Somatic *PIK3CA* Mutation Associated Malignancies and Wilms Tumor

The catalytic subunit of phosphatidylinositol-3-kinase (PI3K) is somatically mutated in many cancers including colorectal, ovarian, breast, hepatocellular carcinomas and glioblastomas. These *PIK3CA* mutations are located mostly at hotspots within the kinase domain (encoded by exon 20), and result in gain-of-function implicated in oncogenicity [Samuels et al., 2004; Ikenoue et al., 2005; Kang et al., 2005]. However, isolated Wilms tumor has not previously been reported in association with somatic *PIK3CA* mutations. The risk of tumorigenesis, including Wilms tumor, in patients with isolated hemihyperplasia ranges from 3.3-6% [Hoyme et al., 1998; Lapunzina 2005; Clericuzio and Martin, 2009]. Wilms tumor has been reported in four individuals with phenotypes associated with somatic *PIK3CA* mutations, including the two described here.

Wilms Tumor Screening Recommendations

Screening has been recommended for young children with syndromic conditions encompassing an increased risk for Wilms tumor, most classically Beckwith-Wiedemann syndrome [Beckwith 1998; Choyke et al., 1999; Clericuzio and Martin, 2009]. Based on the perceived difference in the tumor risk ranging from high in isolated hemihyperplasia to mild or moderate in Klippel-Trenaunay and macrocephaly- capillary malformation syndrome[Table XVIII in Lapunzina 2005], varying recommendations for tumor screening have been proposed [Lapunzina 2005].

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Lapunzina [Table V in Lapunzina 2005] reviewed the screening guidelines for multiple overgrowth syndromes and as expected, the abdominal ultrasound recommendations were identical for isolated hemihyperplasia and Beckwith-Wiedemann syndrome with screening every 3 months until age 4 years, every 6 months until age 7 years and annually thereafter. In contrast, it was recommended that individuals with MCAP receive an annual abdominal ultrasound in all age groups [Lapunzina, 2005]. The American College of Medical Genetics practice guidelines for Wilms tumor screening in patients with isolated hemihyperplasia suggest abdominal ultrasound every 3 months until age 7 years [Clericuzio and Martin, 2009]. No abdominal ultrasound was recommended for individuals with Klippel-Trenaunay syndrome, based on Green et al.'s [2004] review of 115 patients with Klippel-Trenaunay who did not develop Wilms tumor and a study cohort of 8614 individuals with Wilms tumor, none of which had Klippel-Trenaunay. While there was one report each of bilateral Wilms tumor [Ehrich et al., 1979] and bilateral nephroblastomatosis [Mankad et al., 1974] in individuals with Klippel-Trenaunay syndrome, no recent reports of this association have been published. Importantly, these screening recommendations [Green et al., 2004; Lapunzina 2005] were published before the molecular characterization of Klippel-Trenaunay syndrome, and the clinical diagnosis of Klippel-Trenaunay syndrome is not always consistently defined, making it difficult to determine whether reported individuals actually had PROS or another overlapping disorder. Some patients with hemihyperplasia have an underlying somatic *PIK3CA* mutation and their increased risk for Wilms tumor, and possibly other embryonal tumors, may be at least partially accounted for by the *PIK3CA* mutation. Given the prevalence of *PIK3CA* mutations affecting codon 1047 in cancer, a critical consideration is whether patients with these particular mutations are at

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increased risk of Wilms tumors. Including the cases reported here, four individuals with documented *PIK3CA* mutations and Wilms tumor or nephroblastomatosis were reported. The combined number of patients with documented *PIK3CA* mutations in the literature as calculated in Table II and after adding Patient 1 reported here is 258. Considering ascertainment bias for the Patient 1 in this report it is likely that the risk for Wilms tumor or nephroblastomatosis in individuals with *PIK3CA* mutations is less than the calculated 4/258 or 1.6%. While this risk is increased compared to the general population, it does not meet the 2-5% risk suggested by Scott et al. [2006b] in order to warrant screening studies. However, in light of the clearly increased risk and the variable preferences of families and medical care providers in different care environments, we consider screening by ultrasound appropriate, similar to the recommendations for hemihyperplasia [Clericuzio and Martin, 2009], as indicated in Table III. Because many individuals with PROS have overgrowth, the screening guidelines for hemihyperplasia [Clericuzio and Martin, 2009] would be applied prior to the identification of a *PIK3CA* mutation.

Three patients with PROS and Wilms tumor or nephroblastomatosis had somatic mutations affecting PIK3CA codon 1047, which is associated with oncogenicity in isolated cancers. Both patients described here in more detail had extensive overgrowth involving the legs and trunk. Although this evidence is not sufficient to demonstrate high risk, it would be prudent to consider serial abdominal ultrasounds in patients with a somatic *PIK3CA* mutation similar to the recommendations for isolated hemihyperplasia and Beckwith–Wiedemann syndrome. More longitudinal data including clinical examination and regular screening studies are needed on patients with PROS due to different *PIK3CA* mutations, in order to accurately

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determine risk of tumorigenesis. Screening recommendations may then possibly be stratified based on the specific mutation or the clinical presentation.

Acknowledgments

This study was funded in part by the US National Institutes of Health under NINDS grants K08NS092898 (to GMM). KK-N is supported by the Intramural Research Program of the National Human Genome Research Institute. R.K.S. and V.E.R.P. were supported by the Wellcome Trust (grants WT097721 and WT098498), the Medical Research Council (MRC_MC_UU_12012/5) and, with R.G.K., by the United Kingdom National Institute for Health Research Rare Diseases Translational Research Collaboration.

PIK3CA RefSeq NM_006218.2

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Legends:

Figure 1: Ultrasound of Patient 1 obtained at age 9 months, showing multiple hypoechoic, avascular right renal masses in the lower pole and interpolar region (a); contrast enhanced CT scans at age 9 months (b) and 12 months (c) show well-defined low attenuation lesions enlarging in size in the hypertrophied right kidney. The left kidney is atrophied. (c) MRI demonstrates well-defined lesions with mild heterogeneous internal enhancement.

Figure 2: Patient 1's MRI obtained at age 7 years showed (A) a prominent right paraspinal fatty mass involving erector spinae musculature with anterior displacement of the right L3 exiting nerve root implying fat infiltration into the right L3-L4 neural foramen; increased diameter of the right psoas, thigh and calf musculature compared to the left (not shown) with enhancing lesions in the right psoas (B), vastus lateralis and medial right thigh musculature (C), and in the peroneus brevis (D).

Figure 3: (A) Patient 1's back at age 7 years, showing protrusion from lipoma over right lower back, (B, C) Patient 1's legs note overgrowth of right leg, most obvious in the right thigh.

Figure 4: Patient 2 at age 10 years, frontal view (A), showing reduced subcutaneous fat in her face, arms and chest, and lipomatous overgrowth of her left>right abdomen, right leg and left ankle, (B) back view, (C) closer view of her legs with overgrowth of right leg s/p right Syme amputation and lipomatous mass of her medial left ankle, (D) Closer view of her abdomen with masses involving the right upper abdomen, and left mid- to lower abdomen.

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Table I: Phenotypic and Molecular Findings in Individuals with Somatic *PIK3CA* Mutation and Nephroblastomatosis or Wilms Tumor

	Patient 1	Patient 2 [Patient 23 in Keppler-Noreuil et al., 2014]	Patient 3 [CL 2 in Kurek et al., 2012]	Patient 4 [CL29 in Luks et al., 2015]
PIK3CA mutation	p.His1047Arg	p.His1047Leu	p.His1047Arg	p.Asn345Lys
mosaic	yes	yes	yes	yes
Mutation distribution	Blood 0% Psoas muscle 12.9-15.7 % Thigh mass 21.4-24.8%	Blood 0% Left leg adipose & skin 3-4%	Saliva 0% Debulked tissue 20%	314/837 reads from affected tissue
Sex	Male	Female	Female	Female
Onset of symptoms	Birth	Birth	Birth	Not listed
Age at last evaluation	7 years	10 years	Image shown age 18 months	3 years; patient died
Original clinical diagnosis	Hemihyperplasia	Fibroadipose hyperplasia	CLOVES	CLOVES
Asymmetric overgrowth	Yes	Yes	Yes	Yes
Area affected with overgrowth	Right leg	Right foot, leg, buttock; Left ankle, abdomen	Legs, feet	Macrodactyly, wide hands/feet, leg length discrepancy
Fibroadipose overgrowth	yes	yes	yes	Not listed
Area with lipomata	Right lower back, right leg	Left thigh; Right buttock, leg, foot, abdomen	Trunk, limbs	Torso, extremities
Skin findings	Hypopigmented lesion	Pigmented nevus	None listed	Capillary malformation/ lymphatic malformation
Renal findings	Concern for Wilms tumor on imaging studies	Concern for Wilms tumor on imaging studies	Hypoplastic left kidney and Wilms tumor	Renal agenesis/hypoplasia and Wilms tumor
Histology of renal lesion	Post treatment biopsy unable to differentiate between Wilms tumor and nephrogenic rests	Bilateral adenomatous nephrogenic rests	Not listed	Not listed
Treatment	Chemotherapy	Partial bilateral nephrectomy, chemotherapy	Not listed	Not listed
Outcome of renal lesion	Stable after chemotherapy	Stable after chemotherapy	Not listed	Not listed
Other		Lipohypoplasia upper torso, arms and face	Polydactyly	Patient died, no further information listed

Legend: CLOVES; Congenital lipomatous overgrowth with vascular, epidermal and skeletal anomalies

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Table II: Wilms Tumor or Nephroblastomatosis in Cohorts of Patients with Somatic *PIK3CA* Mutations

PIK3CA amino acid change	Seattle cohort	Cambridge cohort	Keppler-Noreuil et al. [2014]	Luks et al., [2015] Table II	Combined number of individuals	Wilms tumor or nephroblastomatosis
	N=86	N=73	N=35	N=73	257 **	
E81K	1	2			3	
R88Q	2	1			3	
R93Q	1	1			2	
R93W	1	1			2	
P104L	1	1			2	
G106V	1				1	
E110del	4	1			5	
G118D		1			1	
N345K				1	1	1
N345T	1				1	
V346_347_Ins_K		1			1	
D350G		1			1	
D350N	1				1	
G364R	1	1			2	
E365K	1				1	
C378Y	6				6	
E418K		1			1	
C420R		2	1	7	10	
P449T	1	1			2	
H450R				1	1	
E453del	2	1			3	
E453K	4	1			5	
P471L		1			1	
E542K	2	8	3	13	26	
E545K	1	7	4*	23	34	
E545D	1				1	
E545G				1		
Q546K				1	1	
Q546R		1			1	
C604R		1			1	
E726K	13	7			20	
G914R	14	2			16	
D939G	1				1	
E970K	1				1	
Y1021C	1				1	
Y1021H	1				1	
T1025A	2	1		1	4	
A1035V	3				3	
A1035T	2				2	
M1043I	7	1			8	

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M1043V		1			1	
N1044Y		1			1	
H1047L		8	8*	6	17	1 (Patient 2)
H1047Y	4				4	
H1047R	2	16	19*	18	51	
G1049R		1			1	
G1049S	2				2	
X1069W	2				2	
Wilms tumor or nephroblastomatosis	0	0	1	1		

*Includes # patients from Cambridge cohort: 1 E545K, 5 H1047L, 4 H1047R. These patients have had follow up screening in the Cambridge cohort.

** This is less than the cohorts combined because 10 patients were included in Keppler-Noreuil et al. [2014] and in the Cambridge cohort.

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Table III: Imaging Recommendations for Patients with *PIK3CA*-Related Overgrowth Spectrum (PROS)

Organ system	Concern or indication	Suggested imaging study	Timing of initial imaging study	Timing of subsequent imaging studies
Brain, Facial	Ventriculomegaly, hydrocephalus, Chiari malformation/cerebellar tonsillar ectopia, cortical brain malformations (polymicrogyria)	Brain MRI without contrast	At diagnosis, if there is macrocephaly (OFC > 2 SD), developmental delay , epilepsy, facial or skull involvement	As indicated based on results of previous studies or when symptomatic
Spinal canal	Tethered cord, Syringomyelia, Lipomeningocele	Ultrasound in infant; MRI thereafter	At diagnosis if truncal involvement present	As indicated based on results of previous studies or when symptomatic
Spine	Scoliosis	Spine radiographs	At presentation if spinal asymmetry or truncal overgrowth is noted	As indicated based on results of previous studies or when new onset scoliosis is suspected
Trunk	For truncal overgrowth, scoliosis, lymphatic or vascular malformations	Whole body MRI, consider contrast as needed	Infants at 12 months (due to need for sedation); for older individuals at diagnosis	As indicated based on results of previous studies or when symptomatic
Extremities	Overgrowth, asymmetry, lymphatic or vascular malformations, thromboembolism	Radiographs, MRI, Consider Doppler ultrasounds of involved arms, legs or both	At diagnosis of overgrowth affecting extremities	As indicated based on results of previous study; to monitor progression of overgrowth or to plan surgery
Kidneys	Enlargement, tumor (Nephroblastomatosis or Wilms tumor),	Renal ultrasound	At diagnosis	Repeat every 3-4 months until age 8 years

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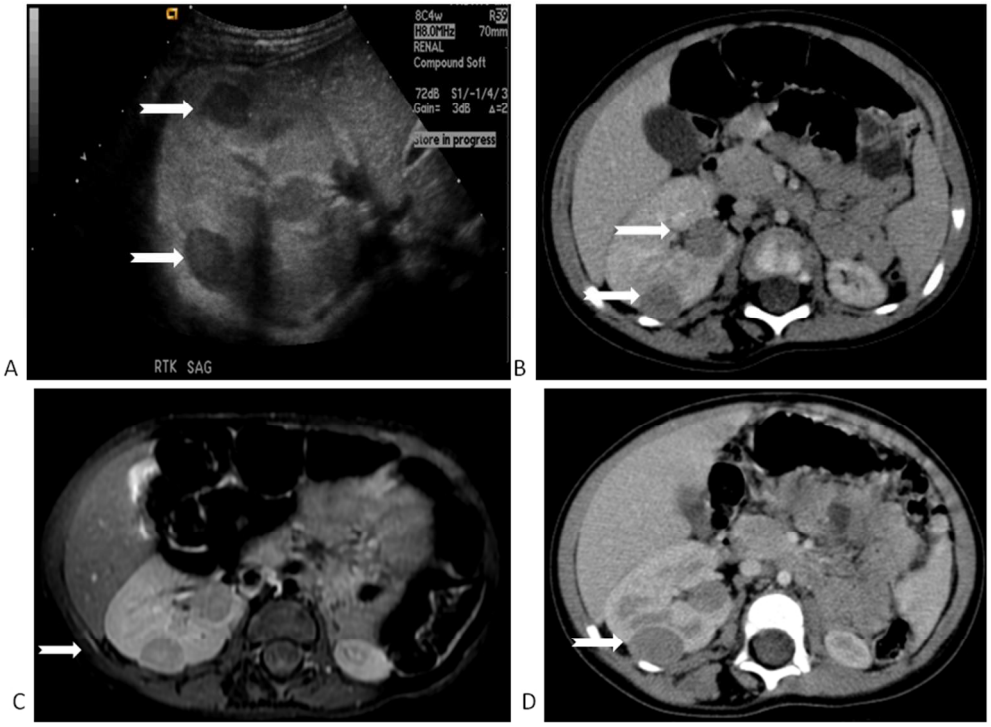

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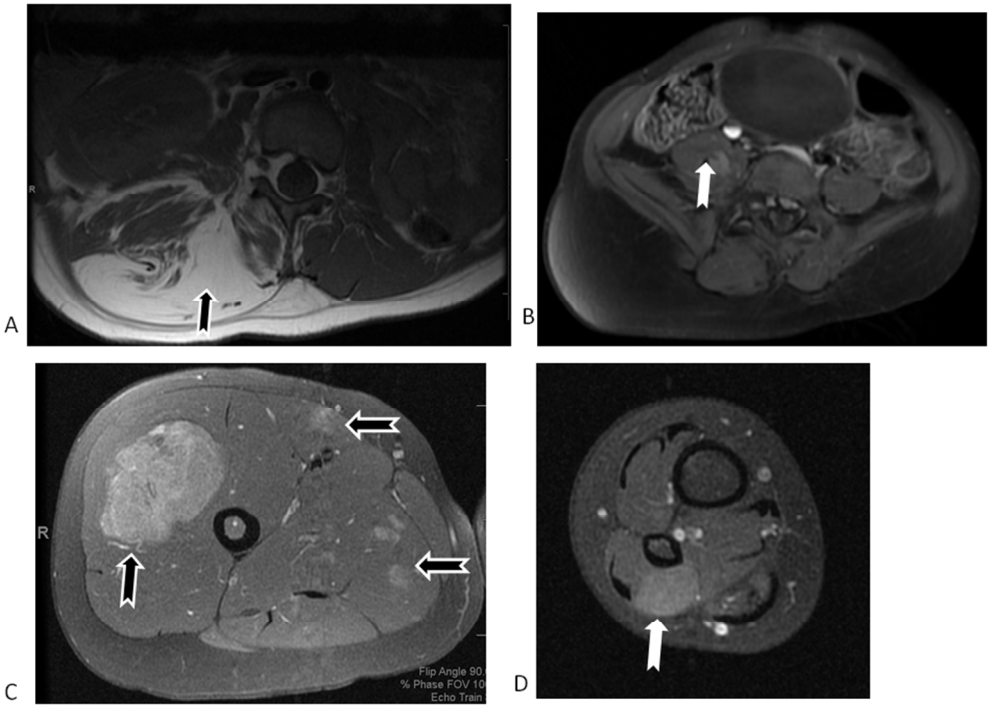
254x190mm (96 x 96 DPI)



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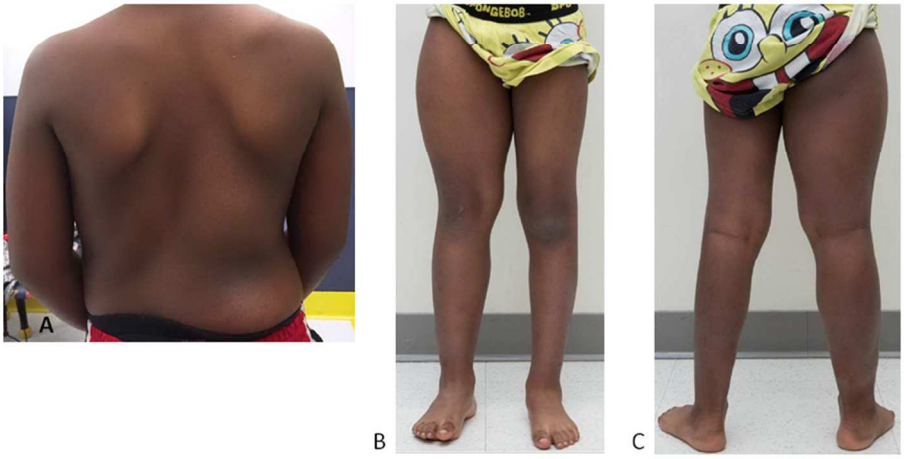

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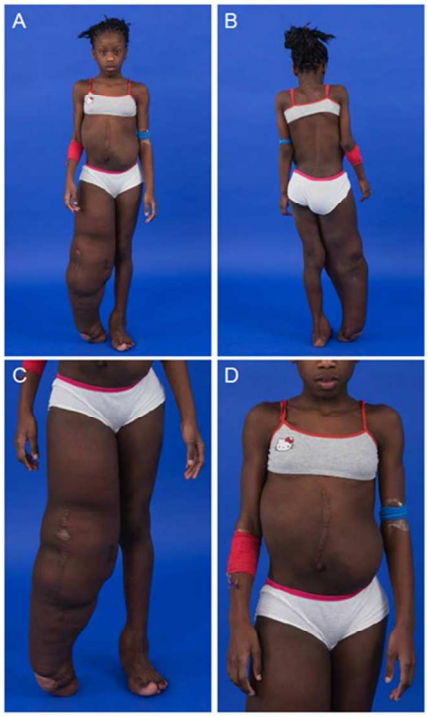

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